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L7: Entry 2 of 66

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TITLE: Method of increasing muscle protein and reducing fat in animals

## BSPR:

The invention relates to neutralization or enhancement of at least one type of endogenous gastrointestinal (GI) neuro-modulator in an animal by administration of antibodies against said GI neuro-modulator or its receptor which increases muscle yield and/or reduces fat in said animal. Although the invention is particularly suited for food animals such as poultry, bovine, ovine, and swine, the invention is applicable to all animals and humans, and particularly those suffering from malnutrition caused by diseases (such as diarrhea, HIV), gastrointestinal disorders, eating disorders and famine.

## BSPR:

In a preferred embodiment, the invention comprises a method of reducing fat and improving meat or muscle yield in an animal which comprises administering to the animal an effective concentration of a specific GI neuro-modulator, and preferably, cholecystokinin (CCK) antibody. In particular, the CCK antibody is produced naturally by immunizing an avian or bovine. The recovered antibodies are transferred naturally to the egg or milk of the avian or bovine, and this antibody containing egg or milk is subsequently administered to the subject animal.

## BSPR:

By administering CCK antibody produced in such a manner, applicants are providing a natural food product for increasing muscle protein and reducing fat in a subject animal without the fear of side effects (excluding, of course, general allergies to eggs or milk). The amount of antibody-containing egg, egg yolk or milk to be added to the feed will vary with the species, size and age of the animal. However, since egg and milk are natural foods and non-toxic, the amount which can be administered is not critical, so long as it is enough to be effective.

## BSPR:

In some cases, an antigen may not be of a sufficient size to effectively or optimally elicit an immune response. In fact, it is generally preferred in the art that a composition having a molecular weight of 10,000 Daltons be used to elicit an immune response. As such, certain modifications must be made to the antigen. For example, isolated CCK peptide has a molecular weight less than 1,500 Daltons. In order to achieve optimal

immunogenicity, it is preferred that the CCK peptide be coupled chemically or through recombinant molecular techniques to larger "carrier" molecules. Examples of "carrier" molecules which make a peptide more immunogenic include ovalbumin, bovine gamma globulin (BGG), keyhole limpet hemacyanin (KLH), mouse serum albumin and rabbit serum albumin, among others. Due to its small size, it is preferred that the CCK peptide be conjugated with a carrier protein having a molecular weight of approximately 8,000 Daltons or more in order to form a conjugate of a size capable of eliciting an immune response.

**BSPR:**

A preferred method of coupling the CCK peptide to a larger protein carrier to form an immunogen is as follows. The CCK peptide is covalently coupled to a purified carrier protein, such as bovin immunoglobulin G (IgG). Electron-microscopy grade gluteraldehyde [O.dbd.CH--(CH.sub.2).sub.3 --CH.dbd.O] is preferably used as a homofunctional coupling reagent, where the aldehyde groups form an irreversible bridge between the N-terminal amino group of the peptide and the available amine groups of the protein carrier molecule. This procedure can be applied as a single step wherein the peptide is simultaneously reacted with gluteraldehyde and bovine IgG in the presence of 10 mM sodium acetate, pH 7. Glycine is then added in order to quench any unreacted aldehyde groups that may still be present. The peptide is then dialyzed and a protein assay is performed to determine the concentration of the peptide. The preparation is then preferably aliquoted and stored frozen.

**BSPR:**

It is preferred that, for purposes of gastrointestinal neuro-modulator neutralization, the target animal either be an egg-producing animal or a milk-producing animal and more preferably, an avian, ovine or a bovine. Avians, ovines and bovines are preferred because they produce an easily administered form of the antibody (i.e. the milk or egg itself). As is well known to those having skill in the art, once an immune response is elicited, antibodies are produced and are transferred to the eggs or milk of the immunized avian or mammal.

**BSPR:**

The dried egg powder can be mixed with food animal feed rations or sprayed directly onto food pellets preferably in oil and thus fed directly to food animals in a simple fashion. Referring to the CCK antibody, typically, 0.1 to 1 CCK antibody-containing egg of this invention is used per 8 pounds of feed. In the case of poultry, spray-dried egg yolk powder is typically sprayed or mixed into poultry feed at 50-500 grams per ton, consistent with maintaining antibody titers sufficient to increase muscle protein and reduce fat in the subject animal.

**DEPR:**

CCK-peptide vaccines were prepared by conjugation of synthetic cholecystokinin (CCK-8) (SEQ ID NO:1) (Fragment 26-33 amide with sulfated tyrosine) to bovine gamma globulin (BGG) using glutaraldehyde. The vaccines were emulsified with Freund's complete adjuvant (1:1) and injected (100 ug CCK) into laying

hens. A second injection of the CCK-8 conjugate in Freund's incomplete adjuvant was injected 7 days after primary injection. A second group of control hens did not receive the CCK vaccination. Approximately 2,880 eggs were collected 5 months after the initial injection and the whole eggs were separated into egg yolk and egg white. The egg yolk was spray dried in 8 lots and the antibody titers of the blended spray dried yolk powder were measured.

DEPR:

Spray dried egg yolk containing anti-CCK-8 antibody with high titers of CCK antibody was blended onto poultry feed and fed to chickens to determine yield efficiency as a result of administering CCK antibodies. The objective of this trial was to determine both individual parts yield and total carcass yield before and after chilling. A field trial was run on 592 Gallus domesticus Broiler type Chickens (Ross.times.Hubbard; Peterson.times.Arbor Acre; Avian.times.Avian). The chickens were of mixed sex, and were started on the feed formulations at the age of one day old hatchlings. A single batch of basal ration for each formulated diet (starter, grower, and finisher) was uniformly mixed. The experimental treatments were mixed as follows: